



# HIGH RATES OF ANTIBIOTIC DRUG RESISTANCE AMONG GRAM-NEGATIVE BACILLI IN LOWER RESPIRATORY TRACT INFECTIONS IN NORTH INDIA

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## ABSTRACT

**Introduction:** Lower respiratory tract infection is a common cause of morbidity and mortality among individuals of all age groups in developing countries. It is caused by wide variety of infectious agents like viruses, bacteria, fungus and parasites. The role of drug resistant bacteria is of most important concern nowadays with fewer antibiotic options available.

**Methods:** A prospective observational study was conducted in an apex referral hospital among patients of all age groups with symptoms of lower respiratory tract infection (LRTI) for a period of 6 months. The samples included respiratory specimens such as sputum, endotracheal (ET) aspirate and bronchoalveolar lavage (BAL). The samples were investigated for bacterial agents by microscopy, culture and anti-microbial susceptibility testing was performed.

**Results:** A total of 525 Gram-negative pathogens were isolated from sputum, ET aspirate and BAL fluid specimens during the study period. In 456/525(87%) cases the infection was mono-microbial in nature and in rest 13% cases the infection was polymicrobial type. The gram negative isolates showed high levels of antibiotic resistance to third generation cephalosporins such as ceftriaxone and ceftazidime which were around 92.1% and 78.9% resistant respectively. The resistance to carbapenems was also high. The maximum susceptibility shown by the isolates was for colistin and tigecycline. Mortality was seen in 13% patients with LRTI.

**Conclusion:** Most of the gram negative isolates obtained from the LRTI samples showed high levels of drug resistance to common antibiotics and showed susceptibility to only high end antibiotics. The findings of the study emphasizes the urgent need for routine surveillance and formulation of antibiotic policies in the hospitals to reduce mortality and morbidity.

**Key Words:** Gram-negative, Antibiotic, Resistance

## INTRODUCTION

Lower respiratory tract infections (LRTI) are one of the commonest diseases among all the age groups which cause high rates of mortality and morbidity worldwide especially in the developing countries like India. LRTI affects the air passages, including the nasal passages, the bronchi and the lungs<sup>1</sup>. LRTI includes range of infections from acute infections, such as pneumonia, pleural effusion, pyopneumothorax, bronchitis to chronic conditions such as asthma, chronic obstructive pulmonary disease (COPD) and tuberculosis. The situation has become even worse with the rising trends of multi-drug resistance (MDR) in gram negative bacterial

infections. Various investigators have advised on judicious use of antibiotics must be done by the clinicians, pharmacist and others who are incorporated in drug delivery system so that a check on the emergence of pathogens acquiring drug resistance to various antibiotics having a role in critical condition or emergency<sup>2</sup>.

The etiological agents of LRTI is diverse in nature. Viruses, bacteria, fungus and parasites all have been implicated as causative agents of the LRTI. Among bacteria Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and Gram-negative bacteria such as *Haemophilus influenzae*, *Pseudomonas*, *Acinetobacter*, and *Kleb-*

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*siella pneumoniae* have been implicated as causative agents of LRTI<sup>3</sup>. The studies have reported that morbidity and mortality due to LRTI can be due to various factors including characteristics of the population at risk, standard of the health-care facilities available, immunosuppressive drugs, inappropriate antibiotic therapy, distribution of causative agents and prevalence of antimicrobial resistance<sup>4</sup>.

With knowledge of local pathogen prevalence and antibiotic the physician can implement the initial empirical antimicrobial therapy which is more promising as per epidemiological data and has greater chances of decreasing the risk of death and morbidities<sup>5,6</sup>. There is paucity of information on the profile of antimicrobial agents among LRTI in our settings. Therefore the present prospective observational study was conducted with the aims to study the etiological agents' distribution causing LRTI and the emergence of resistance among the antimicrobial agents used for treatment of gram negative infections.

## MATERIALS AND METHODS

In the present study, lower respiratory specimens included sputum, endotracheal aspirate and broncho-alveolar lavage fluid (BAL) were collected from patients of all age groups during the period of six months. The samples were collected from patients with clinical suspicion of LRTIs which included fever, presence of purulent sputum on expectoration or positive pulmonary findings on physical examination or radiological findings. The adequate amount of sample was collected in sterile, clean and leak-proof universal container and then transported to the microbiology laboratory within 2 hours of collection. The samples when received in the laboratory were processed immediately without any delay for gram staining, culture and susceptibility testing. The sputum samples which on gram staining showed less than 25 leucocytes and fewer than 10 squamous epithelial cells per field read under oil immersion objective (x100) were rejected for further processing and considered as inadequate and heavily contaminated with oropharyngeal flora containing saliva.

The processing was done in BSL-2 taking all aseptic precautions. After direct gram staining was performed the samples were plated on Blood agar, Chocolate agar and MacConkey agar. The plates after inoculation were incubated at 37°C for 18-24 hours. The morphological, physiological, and biochemical features of the bacterial isolates were determined according to Mackie & McCartney<sup>7</sup>.

Antimicrobial susceptibility testing were performed on significant bacterial isolates using the Kirby-Bauer disk diffusion method, following the recommendations of the Clinical and Laboratory Standard Institute (CLSI, 2015)<sup>8</sup>. Mueller Hinton agar (MHA) was prepared with addition of 5% sheep

blood. A 0.5 McFarland turbidity standard equivalent bacteria suspension for inoculation was prepared and inoculated. Antibiotic disks (Hi-Media) were applied and the plate was incubated at 37°C for 18-24 hours. The following antibiotics were used: Ceftriaxone (30µg), Ceftazidime (30µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Meropenem (10µg), Cefoperazone sulbactam (75 µg +30 µg), Colistin (10 µg) and Tigecycline (15 µg). The strains of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603 were used for assessing quality control. Only one sample from each patient was included in final analysis.

## Statistical Analysis

The Statistical Package for Social Science (SPSS) version 18.0 software was used for the descriptive statistical analysis of the data of the sample population. The frequencies and percentages were calculated for variables under study.

## RESULTS

A total of 525 gram negative isolates were obtained from sputum, ET aspirate and BAL specimens received in microbiology laboratory during the study period of 6 months. 456/492(92.6%) patients had only monomicrobial infection 36/492 (7.4%) had mixed and polymicrobial infection with more than one type of gram negative bacteria recovered from the sample. It was found that *Acinetobacter baumannii* 28.1% (148/525) was most common isolate found in the study followed by *Klebsiella pneumonia* 24.8%(130/525) and *Escherichia coli* was found in about 17.1% (90/525) cases. The gram negative pathogens isolated from patients with LRTI is shown in Table-I.

The majority of isolates were found to be resistant to the third generation cephalosporins like ceftriaxone and ceftazidime. 78.9% of the isolates were resistant to ceftazidime and 92.1% isolates were resistant to the ceftriaxone. Only 45.4% of the gram negative isolates were found to be sensitive to the amikacin. The carbapenem drugs imipenem and meropenem also showed high level of drug resistance 58.6% and 62.9% respectively. Around 84% of isolates were resistant to ciprofloxacin. The only two drugs which showed high sensitivity to the isolates were colistin (97.8%) and tigecycline (99.6%). The susceptibility profile of all the antimicrobial agents is shown in Fig.1. On follow-up it was found that mortality was around 13% and 47 patients (9.5%) had left against medical advice and 77.4% patient were discharged after improvement.

## DISCUSSION

The current study conducted in a referral centre shows that the gram negative isolates were the predominant pathogens

in patients with LRTI. The predominance of *Acinetobacter baumannii* (28.1%), followed by *Klebsiella pneumoniae* (24.8%) and *Escherichia coli* in (17.1%) cases. *Pseudomonas aeruginosa* (12.7%), *Pseudomonas sp.* (10.6%) and *Acinetobacter lwoffii* (2.3%) were also found in significant number of patients with LRTI. This is in contrast to other studies which have shown *Pseudomonas sp.*, *Klebsiella pneumoniae*<sup>5</sup>, *Haemophilus influenzae*<sup>10</sup> as common isolates obtained in such patients. In a multicentric study conducted in 366 patients gram negative etiological agents isolated were *Haemophilus influenzae* (78), *Pseudomonas aeruginosa* (46), *Pseudomonas species* (14) and *Klebsiella pneumoniae* in 21 patients<sup>9</sup>.

In another similar study conducted among 200 patients in India it was found that the gram negative pathogens causing LRTI, in decreasing order, were *Klebsiella pneumoniae* (36.79%), *Pseudomonas aeruginosa* (11.92%), *Escherichia coli* (5.7%) and *Proteus mirabilis* (2.59%) which is similar to our settings<sup>11</sup>. It is interesting to find that the *Acinetobacter baumannii* was the most common isolate which is quite different to the other such studies. The prevalence of pathogenic agents varies across the different geographical regions<sup>12</sup>. Therefore there is need to upgrade the knowledge of the etiological bacterial agents which are causative pathogens in patients with lower respiratory tract infections in a given population so that the trends of bacterial profile can be monitored and this will guide in the empirical drug of choice in a given population and guide antibiotic policy of local region. This will be extremely beneficial for the patients of high risk area and will curtail down the morbidity and mortality in such defined populations. So advantage of the monitoring will be beneficial both for therapeutic and epidemiological purposes.

As per the World Health Organization (WHO) there are high proportions of antibiotic resistance in bacteria that cause common infections especially pneumonia infections in all regions of the world<sup>13</sup>. These patients with LRTI infections caused by drug-resistant gram negative bacteria are generally at increased risk of poor prognosis and death and also responsible for the economic losses<sup>14</sup>. The rise of antimicrobial resistance is of utmost importance in Gram-negative bacteria because we have very few antibiotics for this particular pathogen group<sup>15</sup>. In the present study the rates of resistance of common antibiotics used for respiratory infections showed high level of resistance. The ceftazidime was resistant in 78.9%, ceftriaxone (92.1%), amikacin (52.1%) and ciprofloxacin (84%) cases. In a similar study conducted among 227 patients it was found that most of the bacterial isolates were susceptible to gentamicin (80.9%), meropenem (75%), ceftazidime (62.5%), cefotaxime (57.9%) and ceftazidone (57.9%)<sup>16</sup>. In our study we report even higher burden of resistance than previously reported.

In the recent years several investigators across the globe have reported increase in the antibiotic drug resistant strains and this appears mostly due to several factors like prolonged and inappropriate use of invasive devices and antibiotics<sup>17</sup>. The other factors like high risk sophisticated procedures, immune-suppression and insufficient application of standard and isolation precautions also are attributed to emerging resistance<sup>18</sup>. There is also confusion among the clinicians over the aetiology of lower respiratory tract infections when treating patients when they face limited diagnostic facilities options especially in resource setting conditions. Limited data forces them to use combined therapy for long duration which further worsens the situation and increases the chances of emergence of more drug resistant strains. The first line drugs which were cheap become ineffective and patients are put on high end costly drugs which cause economical loss for the patients<sup>19</sup>. Thus this study gives a guide to physician about gram negative etiological agents and antibiotic resistance profile of antimicrobial agents especially in hospitals which have no antibiotic policy.

## CONCLUSION

The recent years have witnessed the increase in the multidrug resistant strains across the globe especially in the setting of the nosocomial and hospital acquired infections. It leads to increased mortality, prolonged hospital stay, treatment failure, spread of drug resistant strains in the community, selection pressure, threat of pre-antibiotic era and increased burden on the health costs. The emergence of drug resistance is usually multifactorial in nature and affects the patients and the hospital or other health care facility. The rise is mostly attributed to the inappropriate use of antibiotics, understaffing, overcrowding, poor health infrastructure, antibiotic abuse in animals and absence of the local and national guidelines and policies. The increase in MDR isolates from respiratory tract infections is alarming and must be treated with the results based upon the antibiotic susceptibility pattern of the organisms isolated. There is also the need to differentiate the pathogens and colonizers before starting the treatment to prevent further emergence of the MDR strains and it can be done with correlation of the clinical and laboratory parameters.

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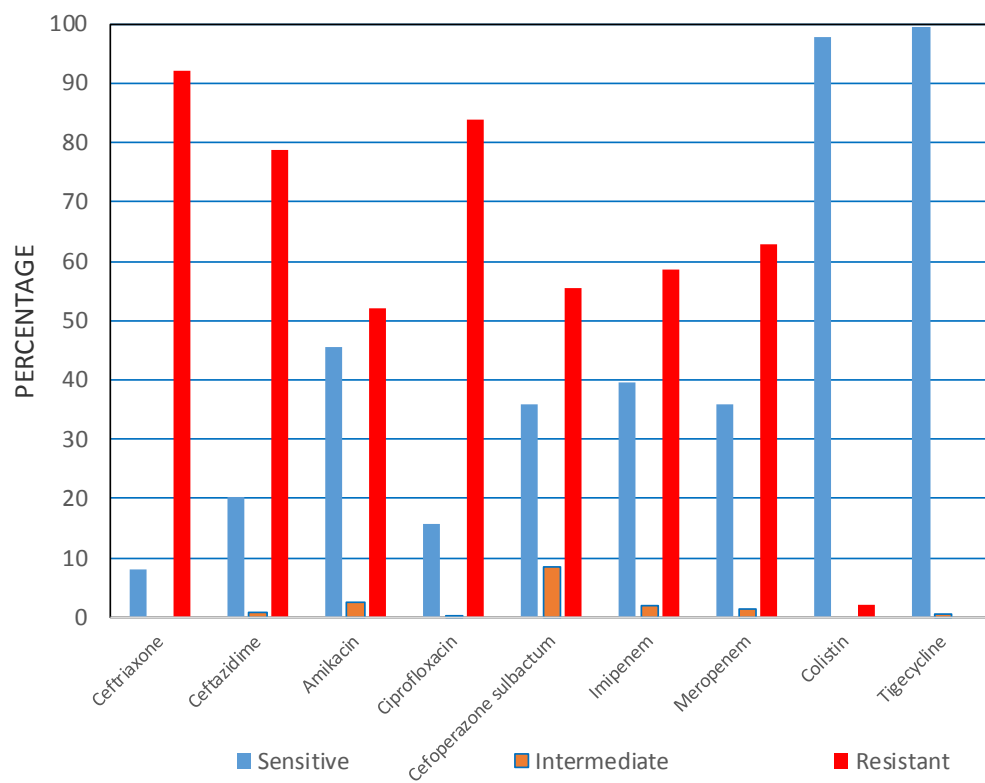
**Conflict of interest:** Nil

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**Table I: Gram Negative Bacteria isolated from the Patients with lower respiratory tract infections (LRTI)**

Gram Negative Bacteria	Total
<i>Acinetobacter baumannii</i>	148
<i>Klebsiella pneumonia</i>	130
<i>Escherichia coli</i>	90
<i>Pseudomonas aeruginosa</i>	67
<i>Pseudomonas species</i>	57
<i>Acinetobacter lwoffii</i>	12
<i>Citrobacter freundii</i>	4
<i>Proteus mirabilis</i>	4
<i>Klebsiella oxytoca</i>	4
<i>Enterobacter aerogenes</i>	3
<i>Citrobacter koserii</i>	3



**Figure 1:** Antibiotic Susceptibility Profile of the Antimicrobial Agents used for Gram Negative Isolates.